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H1 HISTONES OF ADULT TISSUES OF THE SEA URCHIN, *STRONGYLOCENTROTUS PURPURATUS*

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Abstract

Embryonic development of *Strongylocentrotus purpuratus* is characterized by a shift from a faster moving H1 (H1m) found during cleavage stages to a slower moving H1 (H1g) which is synthesized during the blastula and later developmental stages. Sperm have been characterized as having still other H1 histones. The present work describes the H1 histones of adult tissues (coelomocytes, tube feet, intestine, mesentery, testis, and sperm), as revealed by electrophoresis of histone samples in polyacrylamide gels containing sodium dodecylsulfate (SDS). Indications are that sperm possess two subtypes not found in somatic tissues or embryos and that adult tissues possess one H1 subtype not found in sperm or embryos. The possibility that these H1 subtypes represent post-translational modification is discussed.

Introduction

Shifts in the expression of histone H1 gene subtypes are not the result of post-translational modification of the proteins, but are the result of the expression of different sets of histone H1 genes (Ruderman and Gross, 1974; Kunkel and Weinberg, 1978; Hohman, 1980; Harrison and Wilt, 1982). In considering mechanisms of control of histone gene expression, it is of some consequence to be aware of the number of H1 subtype proteins encoded by histone genes which are expressed at different times during development or which are expressed in the adult as tissue specific H1 subtypes. In the sea urchin, the embryos have been far more thoroughly studied than the adults. Brandt *et al.* (1979) describe the H1 components of the adult gut of the sea urchin *Parechinus angulosus.* To date, there are no data available dealing with the H1 histones of the various adult tissues of *S. purpuratus,* though the genes coding for the embryonic histones of this animal have been extensively studied (Cohen *et al.,* 1975; Newrock *et al.,* 1978; Kedes and Maxson, 1981; Maxson *et al.,* 1983).

The investigations reported here deal with the H1 histones of the intestine, tube feet, mesentery, coelomocytes, testis, and sperm of *S. purpuratus.* Histones of plutei and blastulae are included for comparison.

Materials and Methods

Adult *Strongylocentrotus purpuratus* were obtained from live tanks at the Friday Harbor Laboratories of the University of Washington. Adult tissues were dissected from animals whose coelomic fluid had been previously drained using a 50 cc syringe

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fitted with an 18 ga. needle. Intestine and mesentery were distinguished by their pigmentation; mesentery is deep purple, intestine is lighter brown. Tube feet were obtained by cutting out those sections of the test containing the ambulacral system, and scraping the inner surface to remove the inner portions of tube feet. Coelmic fluid was centrifuged to recover the coelomocytes. Testis was obtained as free as possible of spermatozoa by prior KCl injection to induce spawning, and by repeated washing to remove sperm. It proved to be virtually impossible to prepare testis free of contaminating sperm. Sperm cells were also collected for histone extraction. All tissues were washed in cold filtered sea water to remove parasitic ciliates prior to homogenization. Ovary was initially included in the list of tissues to be examined. After processing, it was found that the sample obtained from ovary contained too many contaminating basic proteins, and too little histone to provide a useful comparison with other tissues.

Embryonic stages were obtained by standard fertilization and culture techniques following KCl injection to induce spawning. Histones were extracted from early blastulae and from pluteus larvae.

Following washing, tissues or embryos were washed in 2 changes of a medium containing 0.45 *M* NaCl, 0.025 *M* EDTA, pH 7.4, and 2 changes of a second medium containing 0.075 *M* NaCl, 0.01 *M* EDTA, 0.01 *M* Tris, 0.1 *M* $NaHSO_3$ (TENN). Twenty microliters of TX100 were added per 100 ml of TENN. Tissues and embryos were homogenized in 5 volumes of TENN for 15 seconds in a Waring blender (50 ml cup). The homogenates were examined for unbroken cells using phase contrast optics, and then centrifuged at 2000 rpm for 10 minutes to sediment nuclei. The nuclear pellet was suspended in 2 to 5 ml of TENN, layered over 2 volumes of 1 *M* sucrose in TENN, and centrifuged for 10 minutes at 5000 $\times$ *g* in a Sorvall HB4 rotor at 4°C. The nuclear pellet was suspended in 1–2 ml of distilled water, and extracted with an equal volume of 1 *N* H_2SO_4 for 2 hours, in an ice bath. Histones were precipitated from the supernatant with 2.5 volumes of ethanol overnight, and dissolved in 0.5–1.0 ml of 2% Sodium dodecylsulfate (SDS), 0.0375 *M* Tris, 4 *M* urea, 5% 2-mercaptoethanol.

Electrophoresis was performed using 13.5% SDS-acrylamide gels (75:1 bis acrylamide), and a 4% (75:1) spacer gel (Laemmli, 1970). The electrode buffer consisted of 0.025 *M* Tris, 0.19 *M* glycine, pH 8.4, and 0.1% SDS. Bromphenol blue in 20% sucrose was used as a tracking dye. Gels were run at constant power of 5–6 W (80 V, 75 mA), and were stained with Coomassie brilliant blue R.

Results

Figure 1 shows the resolution of H1 histones of embryonic and adult tissues. The faster moving H2–H4 histones have been intentionally electrophoresed out of the gel in advance of the bromphenol blue front, which remains as an index for estimation of distance migrated by the H1 histones. This procedure permitted migration over a greater distance, with greater resolution of the H1 bands. The extraction procedure removed core histones from isolated nuclei with variable results, especially for H4. The R_f values in Table I are derived from the migration pattern in Figure 1.

Adult tissues all show the same two H1 bands, the slower of which has the same R_f value as embryonic histone H1g. The more rapidly migrating Adult H1-II histone appears distinct from embryonic H1 histones and sperm H1 histones. Testis and sperm show two (slower) H1 bands which are distinct from embryonic H1's and other tissue H1 bands.

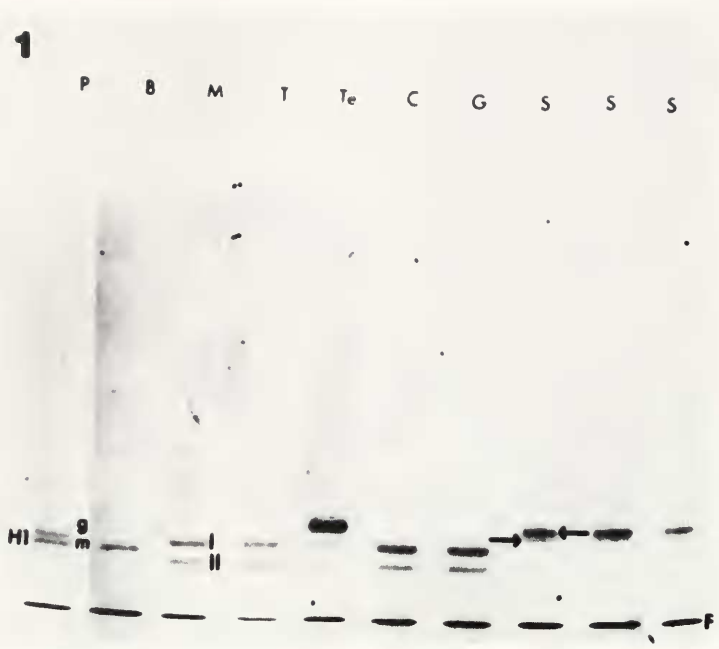

FIGURE 1. Lanes contain 20 μl samples of embryonic stages or adult tissues: Lane 1, P = pluteus. 2, B = blastula. 3, M = mesentery. 4, T = tube feet. 5, Te = testis. 6, C = coelomocyte. 7, G = intestine. 8–10, S = sperm. H1, g & m indicates position of embryonic histones. I and II = positions of adult H1 histones. Arrows indicate H1 histones of sperm. F = bromphenol blue front. Anode at bottom of gel.

DISCUSSION

Some evidence exists to support the notion that the expression of the H1 genes coding for the various subtypes is related to factors regulating cell phenotype in mammalian and sea urchin systems (Hohman, 1980; Arceci and Gross, 1981). For this reason, attention has been focused on H1 histones, as opposed to the more highly conserved core histones.

The present study indicates that at least one H1 variant (subtype) different from those found during embryonic development may be present in adult somatic tissues, and that sperm possess two subtypes not found in embryos or adult tissue. It remains possible that adult H1-II represents a phosphorylated modification of other histones resolved by this system. Charge difference resulting from phosphorylation could be expected to increase mobility in SDS gels. The effects on migration of methylation

TABLE I

Migration (R_f values) of adult and embryonic H1 histones

H1 Histone	n in sample	Mean R_F	S.D.
H1m	2	875	±3.54
H1g	1	856	—
Adult I	5	855	±3.42
Adult II	5	895	±2.19
Sperm I	2	817	±2.83
Sperm II	2	828	±0.00

or acetylation are minimal in SDS gels. Because of their slower migration, the bands resolved in sperm and testis (Sperm H1-I and II) are unlikely to be the result of phosphorylation of H1g or some other H1 co-migrating with H1g. Also, phosphorylation of H1 histone is closely related to cell division, which is absent in sperm. Therefore this possibility seems remote (Easton and Chalkley, 1972).

If the Adult H1-II and sperm H1's do represent valid H1 subtypes, it is evident that histone genes coding for these variants are under control at either the transcription, processing, or possibly the translational level during embryogenesis and spermatogenesis. Testis shares the H1 histones found in somatic tissues, in addition to having the subtypes found in sperm. The presence of a more slowly migrating H1 band in sperm was initially reported by Easton and Chalkley (1972), using acid-urea gels to resolve histones of *Arbacia punctulata.* Those authors concluded that perhaps the sperm H1 subtype is somehow related to the greater degree of compaction of sperm chromatin. H1 histones have been implicated in the superstructure of chromatin by virtue of their position as bridges between nucleosomes (Newrock *et al.,* 1977).

The application of ion-exchange chromatography to the H1 histones of adult sea urchin tissues might well reveal additional tissue specific subtypes, reflecting the various cell phenotypes in specialized tissues.

Brandt *et al.* (1979) reported two H1 bands from the gut of *P. angulosus,* resolved on acid-urea gels. Since the system for resolving the proteins differs from the method described here, the results are not easily compared.

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